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Defensive emotional reactions and stress

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CHAPTER 6

GENERAL DISCUSSION

Summary of the findings

In the first part of this thesis, we examined the presence of dopamine in the dIPAG and determined the origin of this dopaminergic innervation. Our findings indicate that several dopaminergic cell groups project to the dIPAG and that the A13 cell group, located in the medial zona incerta, is the main source of dopaminergic terminals in the dIPAG.

The aim of the second part of this thesis was to investigate the ultrastructural and molecular relations between norepinephrine and glucocorticoids, and the long-term effect of these interactions for anxiety and memory.

In Chapter 3, using immunofluorescence in combination with immuno-electron microscopy and biochemical techniques, we showed that β 2-AR and GR were co-localized in excitatory synapses in the BLA. We also showed that β 2-AR activation by clenbuterol increases the phosphorylation of GR on serine 154, which is a ligand-independent phosphorylation site.

In Chapter 4, we showed that an acute administration of corticosterone could induce a delayed and transient anxiolytic effect that is dependent on noradrenergic activity, but that is not directly linked with HPA axis responsivity.

In Chapter 5, we showed that the same anxiolytic corticosterone treatment could simultaneously impair performance in a high arousing learning and memory task, but not in low arousing task

Dopaminergic projection to the dIPAG, implication for defensive behavior

The PAG controls several functions in which dopamine is known to be involved. However this implication almost exclusively concerns the ventral PAG. Dopamine located in the ventral PAG participates in several functions such as the modulation of opiate-induced analgesia (Flores et al., 2004), the rewarding sensitizing properties of heroin (Flores et al., 2006), or micturition reflex (Kitta et al., 2008). There is little information concerning the dopamine terminals and receptors in the dIPAG which orchestrates defensive reactions. To date, no link has been established between dopamine and dIPAG-mediated panic-like response although there is evidence that dopamine facilitates this type of behavior in areas of the hypothalamic defense system (Maeda et al., 1985; Sweidan et al., 1990 and 1991). But it is legitimate to ask whether dopamine has the same facilitating effects in the dIPAG. One study seems to support the idea. Jenck et al (1990) demonstrated that nomifensine, a dopamine and noradrenaline reuptake inhibitor enhances the aversion induced by electrical stimulation of the dorsal PAG (Jenck et al., 1990). In chapter 1, we traced the origin of the dopaminergic innervation of the dIPAG. We showed that this innervation originates from diverse dopaminergic cell groups. Among these cell groups, the incertohypothalamic cell group (A13) is the main provider of dopamine input to the dIPAG. Considering that this

nucleus projects to the hypothalamic defense system mentioned above (Dominguez and Hull, 2005; Miller and Lonstein, 2009; Wagner et al., 1995), our findings reinforce the hypothesis that dopamine might participate in panic-like response controlled by the dlPAG. Behavioral experiments are needed to confirm or infirm this hypothesis. It is also important to bear in mind that response to imminent threat orchestrated by the dlPAG comprises not only behavior response but also autonomic manifestations that support this emotional and motor reactions. The dorsolateral column of the PAG targets downstream nuclei such as the locus coeruleus, the A5 noradrenergic cell group or the rostral nucleus paragigantocellularis which are involved in arousal, pain processing, cardiovascular and respiratory regulation (Cameron et al., 1995; Van Bockstaele et al., 1993). Dopamine in the dlPAG might be specifically involved in the control of one or more of these functions.

Anatomical and molecular interaction between glucocorticoids and norepinephrine

Strong evidence from human and animal studies has established that norepinephrine and glucocorticoids, through the activation of β -AR and GR in the BLA, play a central role in the modulation of memory consolidation of emotionally arousing information (Joels et al., 2011; Krugers et al., 2011; Quirarte et al. in 1997; Roozendaal et al., 2000 and 2009). It was demonstrated that glucocorticoids potentiate noradrenergic signaling, but on the other hand their effects on memory functions are dependent on this same noradrenergic activity (McIntyre et al., 2002; Quirarte et al., 1997; Roozendaal et al., 2002, 2006a and 2006b). A first model of interaction emerged some years ago to explain the mechanisms of this cross talk between the two stress mediators. In this model, GR and β -AR are localized in a same cell and GR interferes with the β -AR/cAMP/PKA pathway to induce changes on BLA neuron activity, in agreement with earlier studies (Duvarci and Pare, 2007; McGaugh and Roozendaal, 2002) (Fig. 1A). It was then demonstrated that GR also may influence the signaling pathway in a non-genomic manner, suggesting that in addition to their influence on gene expression, glucocorticoids may modulate memory through a membrane associated GR in the synapse (Roozendaal et al., 2002). While the presence of β -AR in synapses is common knowledge, it is only recently that ultrastructural studies have demonstrated the presence of GR at synaptic sites and have provided support for the theoretical mechanisms elaborated (Johnson et al., 2005; Ooishi et al., 2012; Prager et al., 2010). Further support came from physiological and cellular studies to refine the knowledge concerning the interaction of norepinephrine and glucocorticoids, particularly the rapid interaction. Recent findings showed that corticosterone and β -AR agonist rapidly increased AMPA receptor GluA1 subunit phosphorylation and surface expression at the postsynaptic membrane as well as facilitated miniature excitatory postsynaptic currents, whereas the application of each compound alone was less or not effective (Zhou et al., 2012). These results suggest that norepinephrine and glucocorticoids

could mediate the influence of stress on memory consolidation by rapidly altering the glutamatergic neurotransmission. Interestingly, the findings also imply that the receptors of the stress mediators are localized in the vicinity of each other in glutamatergic synapses. The findings from the Chapter 3 demonstrating that β 2-AR and GR are co-localized at excitatory synapses in the BLA confirm this hypothesis and provide the first anatomical evidence of the interaction between norepinephrine and glucocorticoids in this region. The ultrastructural data showed that the two receptors were co-distributed in postsynaptic compartments, supporting the view that GR and β -AR work in concert to influence a post-synaptic response. In addition we also observed co-distribution of the two receptors in the pre-synaptic compartment. The modulation of glutamate release by each hormone separately have already been shown, but there has been no investigation concerning a possible synergetic action at this level, although behavioral data have suggested this idea (Gereau and Conn, 1994; Herrero et al., 1996; Huang et al., 1996 and 1998; Hascup et al., 2010; Reznikov et al., 2007; Venero and Borrell, 1999; Wang and Wang, 2009). It is possible that GR-induced potentiation of β -AR signaling cascade is a general phenomenon involved in different synaptic events, including neurotransmitter release. The presence of the two receptors at the different synaptic compartments supports this view. Therefore in this model, norepinephrine activity is influenced by glucocorticoids resulting in an optimized glutamatergic transmission.

However this model is necessarily incomplete, because it does not include other partners, whereas it is well known that other neuromodulators participate to BLA activity and influence memory functions (Roozendaal and McGaugh, 2011). Recent studies have demonstrated the importance of endogenous endocannabinoids in mediating glucocorticoid effects on memory. Glucocorticoid-induced enhancement of memory consolidation requires the activation of the CB1 receptors by endocannabinoids in the BLA (Campolongo et al., 2009). Endocannabinoids are also required for the non-genomic effects of glucocorticoids on memory retrieval (Atsak et al., 2012a). These findings brought additional complexity to the model. It is now believed that glucocorticoids rapidly enhance endocannabinoid release, which in turn shut down the GABAergic inhibition on BLA pyramidal neurons by activating the CB1 receptors present on interneuron terminals, eventually resulting in increase of pyramidal neurons excitability and also norepinephrine release (Atsak et al., 2012b; Hill and McEwen, 2010) (Fig. 1B). So it seems that the potentiation of noradrenergic activity by glucocorticoids is dependent on endocannabinoid signaling, placing the interplay between the last two at the first place in the sequence of interactions between neuromodulators. But importantly, here again, this interaction seems also dependent on arousal-induced noradrenergic activity (Atsak et al., 2012b; Campolongo et al., 2009; Hill and McEwen, 2009). This suggests that norepinephrine has a primal influence on the cascade that will enhance its activity. In physiological conditions, after a stressful event, neurons are first exposed to high levels of norepinephrine, which is released several minutes before glucocorticoids can reach the brain. Therefore to elucidate the complex stress-induced mechanisms that lead to enhancement of amygdala activity and memory consolidation, it seem logical to first examine the influence of β -AR

activation on glucocorticoid signaling. And if the presence of β 2-AR and GR in the same synapses support the view that GR influence β -AR activity, it also fits with the hypothesis that β 2-AR activity influences GR signaling. We indeed showed that β 2-AR activation modified synaptic GR phosphorylation on serine 154 probably via p38 MAPK mediated mechanisms and formation of a molecular complex that includes the two receptors and p38 MAPK. The phosphorylation site serine 154 (serine 154 in rodents, serine 134 in human) has been discovered recently, and importantly its status is influenced by cellular stress while it is not modified by the presence of glucocorticoids (Gallagher-Beckley et al., 2011). The findings are important because they support the view that norepinephrine may have a critical influence on GR at the very beginning of the stress response, and confirm that the mechanisms of interaction between the two main stress mediators is complex and consists of different steps happening during different phases of the stress response, and probably at different intracellular locations in neurons. These results also explain the behavioral findings showing that noradrenergic activity was necessary for glucocorticoids and endocannabinoids to exert their effects on memory.

The consequences of the phosphorylation on GR serine 154/serine 134 for amygdala activity and eventually modulation of memory need to be investigated. Phosphorylation is an important basic regulator of receptor activity, and all steroid hormone receptors contain multiple phosphorylation sites, which are activated in basal conditions, in response to hormone binding or through specific signaling cascades (Trevino and Weigel, 2013). In their study Gallagher-Beckley and colleagues demonstrated that phosphorylation of serine 134 altered gene expression patterns by modulating promoters' selectivity (Gallagher-Beckley et al., 2011). This clearly shows that phosphorylation of serine 134 is implicated in glucocorticoid genomic effects. But the fact that we showed this particular phosphorylation in synaptoneurosome, suggests that this phenomenon could also be important for the rapid and non-genomic effects. It would be interesting to follow the evolution of the "synaptic" phosphorylated receptors, before the binding to the ligand occurs to examine whether this phosphorylation affects their localization for instance. This would provide information concerning the reason why β 2-AR affects GR phosphorylation status. Does this β 2-AR effect represent a preparation of GR for hormone arrival? Subsequently, it would be insightful to follow those "synaptic" phosphorylated GR after binding to their ligand and compare their spatial and functional evolution with the receptors located in the soma. According to the classical view, binding of ligands to cytoplasmic GR provokes their translocation to the nucleus. But little is known about the GRs located in dendritic spines and synapse for instance. What happens with these receptors once they are bound to their ligand? It is believed that membrane-associated GR exert non-genomic actions, like enhancing endocannabinoid release. An important question is whether the receptors located in the soma and those located in spines constitute two different pools of receptors with different fate and functions? There is a real lack of spatial and temporal information concerning the trafficking of GR.

Many years of behavioral research have detailed the different neurochemical components that mediate stress and arousal influence on memory functions. From the

considerable amount of data accumulated, models have been designed to explain the cellular and molecular mechanisms that underlie this influence of stress on amygdala activity and function. To refine those models, it is critical to design experiments in which the temporal dynamic of the stress response as well as the morphology of neurons involved are integrated.

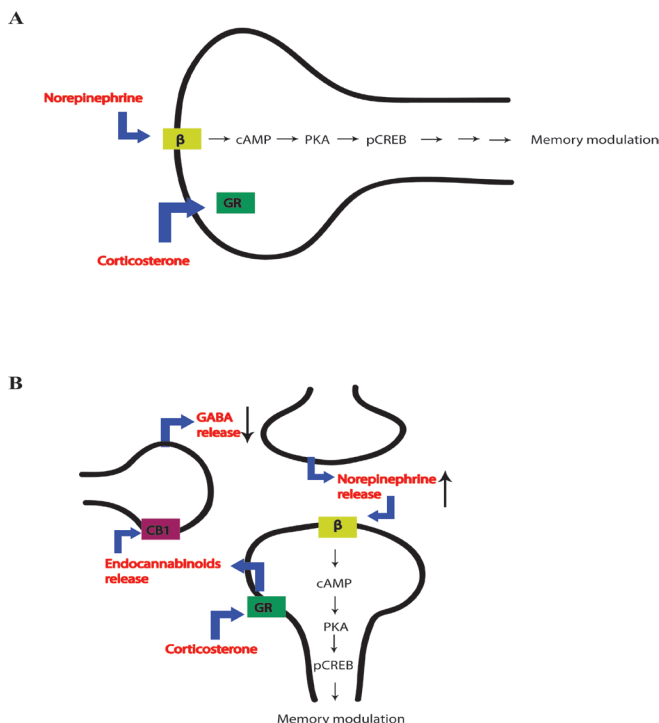


Figure 1. Evolution of the models of glucocorticoid-noradrenergic interaction in the BLA during modulation of emotional memory.

(A) In the old model, the release of norepinephrine during stress or arousal activates the β /cAMP/PKA pathway. Corticosterone facilitates norepinephrine actions by potentiating the activated signaling pathway (adapted from Roozendaal and McGaugh, 2002).

(B) In the current model, binding of corticosterone to a membrane-associated GR induces the rapid release of endocannabinoids. The endocannabinoids bind to CB1 receptors located on GABAergic terminals and inhibit the release of GABA. As a consequence, the inhibition on noradrenaline release is suppressed and the β /cAMP/PKA/pCREB pathway is enhanced (adapted from Atsak et al., 2012).

Mechanisms of glucocorticoids long-term effects on anxiety and memory

It is well established that in addition to promoting a rapid and adequate response to an acute stressful situation, stress hormones also promote long-term adaptation. These adaptive changes prepare the individuals for subsequent stressful events. As mentioned earlier, enhanced memory consolidation of stressful or emotionally arousing information is one of the long-term adaptive responses to stress. For instance, remembering the location where a danger was encountered in the past would help an

individual, by avoiding this or similar locations, to increase their chance of survival. Anxiety behavior, as a normal stress response, also undergoes long-term modifications. Many studies have focused on the long-term maladaptive consequences of stress, and particularly chronic or severe stress, but it seems that stress hormones can also have long-term beneficial effects on anxiety levels (McEwen, 2007). In Chapter 4 we demonstrated that acute treatment of high dose of corticosterone reduces anxiety-like behavior in the elevated plus maze 10 days later. This delayed effect on anxiety is consistent with previous studies demonstrating that acute stress or acute administration of corticosterone can induce changes in anxiety levels several days after (Mitra et al., 2005; Mitra and Sapolsky, 2008). More interestingly, the anxiolytic properties of our corticosterone treatment are in line with evidence showing that glucocorticoids can have beneficial effects in PTSD patients (Aerni et al. 2004; Schelling et al., 2001, 2004 and 2006; Weiss et al., 2006; Yehuda et al., 2010). Glucocorticoids are also well known to decrease anxiety in stress-sensitive individuals or in stressful situations, consistent with the view that these hormones suppress the stress response (Het et al., 2012; Putman and Roelofs, 2011; Rao et al., 2012; Sapolsky et al., 2000; Soravia et al., 2006). However, there were contrasting findings concerning the effects of glucocorticoids in baseline conditions (Andreatini and Leite, 1994; Buchanan and Lovallo, 2001; File et al., 1979; Grillon et al., 2011; Reuter, 2002). The findings of Chapter 4 confirm that in basal conditions, glucocorticoids can be anxiolytic. The reasons for these discrepancies are not clear. For instance, our results are in contradiction with another study in which the same dose of corticosterone caused an anxiogenic effect at the same time point (Mitra and Sapolsky, 2008). It is impossible to compare the baseline level of arousal of animals coming from two different research groups, but an explanation could be that in our experimental conditions, the animals were stressed during the injection and that corticosterone administration suppressed this stress. However we used controls that allow us to rule out this hypothesis. In addition to the vehicle control group, there was a home-cage control group. The two groups displayed comparable levels of anxiety, indicating that the injection did not cause significant stress that could be reversed by corticosterone as it was demonstrated recently by Rao and colleagues (Rao et al., 2012). Besides, habituation sessions before the elevated plus maze test indicated that basal level of arousal of all the animals was not excessive, since those sessions enable a further decrease of anxiety-like behavior in the maze for all the groups while preserving the difference between them. Another explanation would be that the animals were relatively stressed before the injection took place. In that case, corticosterone could act to exert its stress suppressing effects on the long run, which will be reflected 10 days later in the plus maze. This would be consistent with recent evidence indicating that glucocorticoids effects on BLA neurons response depend on stress history (Karst et al., 2010). As mentioned in Chapter 4, we did not assess the morphology of BLA principal neurons in contrast to the study of Mitra and Sapolsky. But examination of the morphological consequences of our drug treatment will be critical to understand how corticosterone can result in opposite emotional states. It appears that the intrinsic activity of important regions such as the BLA, although not directly visible at the behavioral level, play an important role on the outcome of stress

hormone effects.

The effects on anxiety-like behavior were associated with effects on learning and memory performance. In Chapter 5, we demonstrated that the same corticosterone treatment that was able to induce anxiolytic effects was also capable of impairing the performance on a learning and memory task that was high arousing, whereas it had no effect on more neutral task. It has long been known that anxiolytic compounds also affect learning and memory functions that have strong emotional components (Beuzen and Belzung, 1995; Leong et al., 2012; Ribeiro et al., 1999; Uzun et al., 2010). This is not surprising considering that memory functions and anxiety share common neurochemical and neuroanatomical substrates (Kalueff, 2007). Action of glucocorticoids on BLA morphology and synaptic plasticity could be the cause of dysfunctions in emotional memories processing. There is evidence that the integrity of the amygdala is necessary for those types of memories (LaBar and Cabeza, 2006; Richardson et al., 2004; Vuilleumier et al., 2004). This could explain why corticosterone-treated animals perform less than the control animals in the inhibitory avoidance task, and why there is no difference in the object recognition task.

It is also possible that memory functions were intact and that the impaired performance were due the low level of anxiety. The influence of anxiety on memory performance is well documented, and it appears that the link between the two is quite complex (Diamond et al., 2007; Herrero et al., 2006). According to the Yerkes-Dodson law, the effect of anxiety on performance depends on the complexity of the learning task (Broadhurst, 1957; Easterbrook, 1959). Additionally, the emotional component of the task is important as well. Indeed, a minimum level of arousal is necessary to be able to learn and later remember any information (Okuda et al., 2004). Those parameters, together with the emotional state of the subject undergoing the task, are integrated in a complex set of interactions that will influence the performance. An optimal level or awareness, which will be specific for each context, is necessary so that learning and memory formation occur (Silva and Frussa-Filho, 2000). In our experiments, the level of anxiety caused by the corticosterone treatment did not disturb memory processing during the object recognition task, so the treated animals were capable of learning and recalling the information. In contrast, in the inhibitory avoidance task, the level of anxiety may have been too low to enable an optimal processing and/or recalling of the information.

PTSD is a good example of the interconnection between anxiety state and memory processes. A high level of anxiety and frequent re-experiencing of the traumatic event characterize this disorder. The initial failure of the neuroendocrine system to terminate the stress response cause an enhance consolidation of the traumatic memory. The high levels of stress hormones contribute to the retrieval and reconsolidation of this memory. As a result of the constant re-experiencing of the traumatic event, patients develop a permanent and high state of anxiety. This high level of anxiety will also influence future memory processes since it is well known that PTSD patients have memory disturbances (Bremner et al., 1993; Isaac et al., 2006; Samuelson, 2011). Whether the results observed in the inhibitory avoidance task are due to dysfunction of memory processes or an insufficient level of anxiety, they are interesting in the perspective of

PTSD treatment. Glucocorticoids beneficial effects on PTSD symptoms are believed to weaken fear memory (Aerni et al., 2004; De Quervain et al., 2009). It appears from our findings that glucocorticoids can alter both anxiety behavior and emotionally arousing memory, giving to this hormone a double efficiency to treat patients.

While the importance of this interaction was well established for learning and memory functions, less was known about their association for the modulation of anxiety, although there has been strong evidence that both norepinephrine and glucocorticoids play a role in the regulation of anxiety. In the case of memory, this crosstalk is involved in the elaboration of an optimal response. It would be logical that it also affects anxiety behavior, which is also part of the stress response. The finding that corticosterone requires β -AR activity to induce its delayed and long lasting effects on anxiety behavior indicate that the interaction between norepinephrine and glucocorticoids is a general mechanism that modulate other functions than memory processing. The simultaneous alteration of anxiety-like behavior and performance in arousing memory task support this view. Finally, the fact that these alteration occur several days after the administration of the drugs suggests that in addition to modulate immediate reactions to stress, the interaction between the two stress mediators is also involved in generating long-term changes that will be important for both anxiety response and memory functions during future stressful situations.

BLA as a locus of integration and modulation

By activating specific signaling pathways, membrane ion channels, glutamatergic and GABAergic receptors, norepinephrine and glucocorticoids modify the ongoing synaptic transmissions, and norepinephrine is believed to enhance the signal-to-noise-ratio in order to strengthen processing of information that are important for the stress response (Marzo et al., 2009; Roozendaal et al., 2009). In the amygdala, norepinephrine exerts various effects, both excitatory and inhibitory (through β -AR and $\alpha 2$ -AR respectively) on synaptic transmission (Ferry et al., 1997; Gean et al., 1992; Huang et al., 1996). It also promotes the initiation of long-term potentiation and suppresses GABAergic inhibition on amygdala principal neurons (Ikegaya et al., 1997; Tully et al., 2007). Stress levels of glucocorticoids enhance pyramidal neurons intrinsic excitability and suppress GABAergic transmission (Duvarci and Pare, 2007). The two hormones have many common end points, such as the AMPA receptors, the small-conductance Ca^{2+} -activated K^{+} channels (SK channels) which are implicated in the formations of emotionally arousing memories and the regulation of anxiety (Boyle et al., 2013; Faber et al., 2005 and 2008; Krugers et al., 2012; Mitra et al., 2009). In physiological conditions, the interaction and synergetic actions of the two stress mediators in the BLA are implicated in the attribution of emotional value to the sensory information arriving from the cortex and the thalamus, and allow the BLA to modulate diverse behaviors and functions through its direct and indirect connections with the hippocampus, the cortex, the hypothalamus or the brain stem (Pape and Pare, 2010; Sah et al., 2003). We have hypothesized that BLA is involved in the phenomena

observed in Chapters 4 and 5. There is strong evidence that corticosteroids influence on anxiety is correlated with alteration of BLA neurons morphology (Mitra and Sapolsky, 2008). The BLA is also the site that integrates stress hormone signaling to modulate memory functions (Roozendaal et al., 2009). In addition, considering that the defense response is initiated in the BLA via the detection of threat, we can also consider that the dIPAG-induced defensive behavior mentioned in Chapter 1 is also indirectly modulated by BLA activity.

Concluding remarks

This thesis aimed at examining the mechanisms of norepinephrine and glucocorticoids interaction in the BLA and their long-term consequences for anxiety behavior and learning and memory process. The findings provide new information that may be relevant in the study of anxiety disorders such as PTSD, as cortisol is increasingly viewed as a potential therapeutic agent in treating this pathology. However, it is important to keep in mind that all those experiments have been done in male rats, like in most behavioral studies, while it is well established that male and female respond differently to stress (Bangasser and Valentino, 2012). Clinical studies have consistently reported gender differences in the prevalence of anxiety disorder. For instance, it is estimated that women are twice as likely to suffer from PTSD (Bangasser and Valentino, 2012). Evidence showed that these gender-related differences have a biological basis. The HPA axis and the major hormones of the stress system are modulated by ovarian hormones (Olff et al., 2007). In human, HPA response of women between puberty and menopause is different than in men and also change according to the phase of the menstrual cycle (Olff et al., 2007). The noradrenergic and glucocorticoid systems are influenced by female hormones, even at the receptor level (Bangasser et al., 2011 and 2013a,b; Etgen et al., 2001). This suggests that the mechanisms proposed for β 2-AR and GR in the present thesis might be altered in female according to the phase of the cycle. This may have also long-term implications for anxiety behavior as well as processing of emotional information. It would be interesting to integrate the complexity of female hormones system in future studies investigating the cellular and molecular mechanisms of stress hormones interactions. This could provide critical information to explain the gender differences observed in stress response both in pathological and non-pathological situations.

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Nederlandse Samenvatting

Een stressor is een fysieke of psychische stimulus die de homeostase van een organisme bedreigt. In reactie op de aanwezigheid van een stressor, en om het evenwicht te herstellen, maakt het organisme gebruik van fysiologische en gedragsmechanismen. Op gedragsniveau wordt het type respons bepaald door de fysieke afstand tot de dreiging. Dit proefschrift bestaat uit twee onafhankelijke delen, die zich bezighouden met twee verschillende emotionele reacties: paniekerig defensief gedrag in reactie op dreigend gevaar; en angst, hetgeen de reactie op afstand en onvoorspelbare bedreigingen vormt.

Het eerste deel van het proefschrift richtte zich op het dorsolaterale periaqueductale grijs (dIPAG) dat een cruciale rol speelt bij de controle van defensief gedrag in reactie op directe en acute fysiologische dreiging. In hoofdstuk 2 onderzochten we de projecties van verschillende dopaminerge cel groepen naar het dorsolaterale periaqueductale grijs (dIPAG). Deze studie toonde aan dat het dIPAG dopaminerge afferente vezels van verschillende cel groepen, enerzijds gelokaliseerd in de hypothalamus en anderzijds in de ventrale middenhersenen, ontvangt. Van de verschillende dopaminerge cel groepen, werd gevonden dat de incertohypothalamische A13 cel groep de belangrijkste bron van dopaminerge efferenten naar het dIPAG is. Aangezien bekend is dat de A13 cel groep naar hypothalamische gebieden projecteert waar dopamine defensieve reacties faciliteert, ondersteunen deze bevindingen de hypothese dat dopamine dIPAG-gemedieerd defensief gedrag zou kunnen beïnvloeden.

De neuro-endocrienestressrespons bestaat uit de activatie van het autonome zenuwstelsel en de hypothalamus-hypofyse-bijnierschors (HPA)-as, die respectievelijk leiden tot het vrijkomen van noradrenaline en de aanwezigheid van een hoge concentratie van glucocorticoïden in de hersenen.

Het tweede deel van dit proefschrift onderzocht de anatomische en moleculaire basis van de interactie tussen noradrenaline en glucocorticoïden, in de basolaterale amygdala (BLA), en de implicatie van deze interactie voor angst en geheugen.

Hoewel voldoende bewijs het belang van de samenspraak tussen deze twee mediators voor geheugenconsolidatie van emotionele informatie heeft aangetoond, zijn de precieze neurale mechanismen die ten grondslag liggen aan deze interactie niet bekend. In hoofdstuk 3 onderzochten we de ultrastructurele verdeling van de β 2-adrenerge receptoren (β 2-AR) en glucocorticoïd receptoren (GR) in de BLA, met een focus op synaptische sites. We toonden aan dat β 2-AR en GR gecolokaliseerd waren in BLA-synapsen. Verder onderzochten we een mogelijk functioneel gevolg van deze colokalisatie en vonden dat activatie van β 2-AR de hyperfosforylering van GR op haar unieke ligand-onafhankelijke fosforyleringsplaats induceert. De bevindingen geven aan dat deze β 2-AR-geïnduceerde hyperfosforylering van GR wordt gemedieerd door de vorming van een moleculair complex met β 2-AR, GR en het kinase p38 MAPK.

Deze bevindingen bieden niet alleen anatomisch bewijs van de interactie tussen noradrenaline en glucocorticoïden binnen de synaps, maar ze veronderstellen ook een

mechanisme van interactie waarin noradrenaline-signalering GR kan beïnvloeden, zelfs voor de aankomst van de hormonen in de amygdala.

In hoofdstuk 4 onderzochten we de effecten van glucocorticoïden op angstig gedrag en HPA-as reactiviteit en de temporele dynamiek van deze effecten. De resultaten toonden aan dat een enkele toediening van corticosteron vertraagde en omkeerbare anxiolytische effecten onafhankelijk van de HPA-as induceerde. We toonden ook aan dat noradrenaline activiteit noodzakelijk was voor de glucocorticoïden om de angsttoestand veranderen. Deze resultaten tonen aan dat vergelijkbaar met wat gebeurt bij geheugenmechanismen, noradrenaline en glucocorticoïden samenwerken om angstig gedrag te moduleren.

De bevindingen uit hoofdstuk 5 toonden aan dat samen met de tijdelijke anxiolytische effecten waargenomen in Hoofdstuk 4, glucocorticoïden de prestaties in een “high-arousing” geheugentaak verminderden terwijl ze geen effect in een “milde-arousing” taak hadden. Deze gegevens suggereren dat glucocorticoïden tegelijk angst en geheugen beïnvloeden, waarschijnlijk via een modificatie van het amygdala-neuron morfologie en activiteit.

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